



Review

Engineering pancreatic tissues from stem cells towards therapy

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ABSTRACT

Pancreatic islet transplantation is performed as a potential treatment for type 1 diabetes mellitus. However, this approach is significantly limited due to the critical shortage of islet sources. Recently, a number of publications have developed protocols for directed β -cell differentiation of pluripotent cells, such as embryonic stem (ES) or induced pluripotent stem (iPS) cells. Decades of studies have led to the development of modified protocols that recapitulate molecular developmental cues by combining various growth factors and small molecules with improved efficiency. However, the later step of pancreatic differentiation into functional β -cells has yet to be satisfactory *in vitro*, highlighting alternative approach by recapitulating spatiotemporal multicellular interaction in three-dimensional (3D) culture. Here, we summarize recent progress in the directed differentiation into pancreatic β -cells with a focus on both two-dimensional (2D) and 3D differentiation settings. We also discuss the potential transplantation strategies in combination with current bioengineering approaches towards diabetes therapy.

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Abbreviations: 2D, two-dimensional; 3D, three-dimensional; BMP, bone morphogenic protein; ES, embryonic stem; FGF, fibroblast growth factors; IBMIR, instant blood-mediated reaction; ILV, indolactam V; Ngn3, neurogenin 3; PEG, polyethylene glycol; PI3K, phosphatidylinositol-3 kinase; PIPAAm, poly-N-isopropylacrylamide; PVA, polyvinyl alcohol; Pdx1, pancreatic and duodenal homeobox 1; Ptf1a, pancreatic transcription factor 1a; VEGF, vascular endothelial growth factor; iPS, induced pluripotent stem.

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1. Introduction

Pancreatic β -cells are responsible for producing the only hypoglycemic hormone, namely insulin. Whole pancreas or pancreatic islet transplantation is a radical treatment for severe diabetic patients, mostly due to the lack of pancreatic β -cells. However, the number of donors has never been enough, and a possible solution to this problem is regenerative medicine.

Generally, it is difficult to expand adult derived pancreatic β -cells *in vitro*. To provide alternative cells for transplantation, many attempts have been made worldwide to develop a method for inducing the self-renewing multipotent stem cells into functional pancreatic β -cells. Among the many types of stem cells, two types of pluripotent cells are of particular interest in the development of such methods, the ES cells, which are derived from embryos that developed from fertilized eggs, and the iPS cells, which are derived from the reprogramming of human skin cells and other cells. Strategies for directed differentiation into β -cells mostly rely on aspects of physiological development of endocrine pancreas.

This article outlines pancreatic development and differentiation and explains methods for the differentiation of ES and iPS cells into pancreatic β -cells. Furthermore, future tasks are discussed based on the findings of our experiments and the role of bioengineering technologies in regenerative medicine.

2. Pancreatic development (Fig. 1)

The pancreas is an organ of endodermal origin that functions as an exocrine gland secreting digestive enzymes into the duodenum and also as an endocrine gland secreting blood sugar-regulating hormones into the blood. Pancreas initially develops by budding from the embryonic endoderm at the junction of foregut and midgut. Pancreas development is very elaborate and involves specific time- and space-dependent activation of transcription factors and signaling molecules [1–4]. Pancreatic development in mice starts at embryonic day 8.0 upon induction of pancreatic progenitor cells from pancreatic and duodenal homeobox 1 (Pdx-1)-positive cells in the foregut endoderm [5–10] (Fig. 1a). Then, humoral factors such as activin and fibroblast growth factors (FGF) are secreted

from the notochord, upon which pancreatic progenitor cells proliferate to form both the dorsal and ventral buds (Fig. 1b) [11–15]. Activin, FGF as well as retinoic acid [16,17] signaling was demonstrated to inhibit sonic hedgehog homolog, thereby, providing a critical signaling cue for the initiation of pancreatic fate specification [12,18,19]. Later on this pre-patterned pancreatic endoderm develops and differentiates into insulin producing cells (Fig. 1c). Lammert and colleagues [20–22] showed that the removal of aortic endothelial cells impaired pancreatic differentiation in *Xenopus* embryos as well as enhanced differentiation by introducing ectopic vascularization in mice, suggesting important role of endothelium for endocrine pancreas differentiation. Later study using mouse suggested that endothelial cell instruction can be waived in the initiation of Pdx1 expression in endoderm, however, they are essential for emergence of dorsal pancreatic buds and maintenance of Pdx1 expression through the crucial pancreatic transcription factor 1a (Ptf1a) induction [23]. The components of buds are pancreatic progenitor cells that differentiated from endodermal epithelial cells. Even after bud formation, these cells, surrounded by a mesenchyme, continue dividing so that buds can further grow symmetrically. Understandings of epithelial–mesenchymal interactions between pancreatic progenitor cells and pancreatic mesenchymal cells during the above process are extremely important in recapitulating pancreatic development in culture. At embryonic day 14.5, the ventral bud moves to lie on the dorsal side of the dorsal bud upon rotation of the stomach and duodenum, and the two buds eventually fuse (Fig. 1d). This is followed by pancreatic duct formation, and the ductal network formation starts upon branching. In parallel to the pancreatic duct formation, the duct cells give rise to pancreatic α and β -cells, and mature islets with a core-mantle structure are formed immediately prior to birth (Fig. 1e). The induced β -cells produce vascular endothelial growth factor (VEGF), then attract blood vessels, and potentiate insulin expression by recruiting a denser vasculatures compared with surrounding exocrine components [20]. After birth, mesenchymal cells differentiate into perivascular cells around the vascular networks of the islets, thereby contributing to the long-term stability of the vascular networks. In addition, vascular endothelial cells are in a close relationship with the islet endocrine cells throughout the

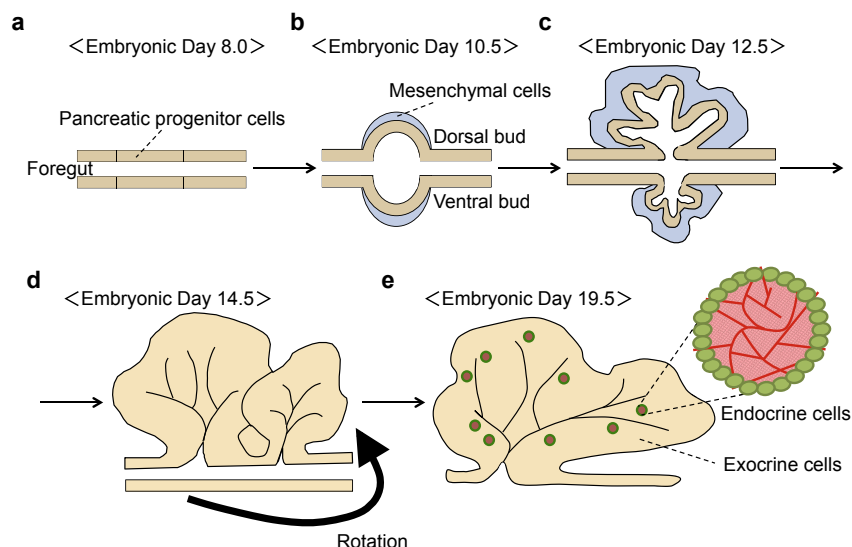


Fig. 1. Mouse pancreas development and endocrine cell differentiation.

Table 1
Comparison of existing strategies for insulin-positive cell production.

1st Author (year)	Stem cell	Culture method	Glucose tolerance test <i>in vitro</i>	Glucose tolerance test <i>in vivo</i>	Glucose normalization <i>in vivo</i>
Lumelsky N (2001)	mES	2D	++		+
Afrikanova I (2002)	mES	2D	++	++	++
D' Amour (2006)	hES	2D			
Kroon E (2008)	hES	2D		+++ (C-peptide)	
Bernardo AS (2009)	mES	2D	+		
	hES				
Hrvatin S (2014)	mES	2D			+++
	hES				
Alipio Z (2010)	miPS	2D	+		+++
Jeon K (2012)	miPS	2D	+		+++
Tateishii K (2008)	hiPS	2D	+	(C-peptide)	
Kunisada Y (2012)	hiPS	2D	++	(C-peptide)	
Thatava T (2013)	hiPS	2D	+	(C-peptide)	
Wang XI (2009)	mES	3D	+++	(C-peptide)	
Saito H (2011)	hiPS	3D	++		++
Pagliuca FW (2014)	hES	3D	+	+	+++
	hiPS				
Toyoda T (2015)	hES	3D		+(C-peptide)	
	hiPS				
			+++ (10-fold)		+++ (Blood glucose level <200 mg/dl)
			++ (5–9-fold)		++ (Blood glucose level >200 mg/dl)
			+(2–4-fold)		+(Maintaining body weight)

entire process, including early islet development and the initiation of function of the matured islets [20,23,24]. Signals from the blood and paracrine signals from the epithelial cells play important roles in various events, such as endocrine cell differentiation, islet development and enhancement of its function. The diversity and importance of angiocrine signaling indicates that interactive development of pancreas and its vasculature is a general phenomenon. Different steps of embryonic pancreas development as well as their post-natal function require inductive signals from endothelial cells. Many studies have demonstrated induced differentiation into pancreatic β -cells by mimicking such molecular mechanisms essential for the individual steps of pancreatic development. However, there are lots of unknown events potentially regulating whole pancreatic developmental process beyond simple protein secretion events, which can be learned from genetically modified animals. For instance, it would be fascinating know how the early hierarchical structures emerge as a result of region specific multi-cellular interaction through direct or indirect process. In this way, notable advances in 4-D imaging and gene-editing approaches will open a new possibility to study such previously uncharacterized process of pancreatic development.

3. Generation of pancreatic β -cells

3.1. Methods for the induction of the differentiation of ES cells into pancreatic β -cells

ES cells are pluripotent stem cells derived from the inner cell mass of a blastocyst. Mouse ES cells injected into the blastocyst cavity differentiate into any type of cell, including germ cells. This signifies that diabetes could become curable once pancreatic β -cells that are differentiated from ES cells by *in vitro* induction systems become available for transplantation.

The directed differentiation of ES cells into pancreatic β -cells (insulin-producing cells) has been demonstrated in many studies summarized in Table 1 [25]. One earliest example of a successful approach via nestin-positive cells was reported by Lumelsky et al. [26], wherein mature insulin-producing cells were differentiated from mouse ES cells via five step directed differentiation protocols. The study was followed by a series of reports that demonstrate the induced differentiation of ES cells into insulin-producing cells [27–29]. For instance,

Hori tested a similar approach [30] and successfully induced differentiation into pancreatic β -like cells by adding a phosphatidylinositol-3 kinase (PI3K) inhibitor at the last stage of differentiation. It was reported that the resulting cells formed islet-like structures and produced more insulin than those in previous reports.

The overexpression of Pdx-1, which is a common marker of pancreatic progenitors and β -cells, has also been widely used to augment pancreatic differentiation [29,31]. Briefly, exogenous Pdx-1-VP16 fusion proteins that translocated into the nucleus at certain times in the early and late stages of differentiation induction were functional, resulting in the effective production of insulin-producing cells from mouse or human ES cells. The expression levels of insulin, Pdx-1, GLUT2 and C-peptide mRNAs were high in those cells. These studies improved the efficiency of differentiation into insulin-producing cells, but there are problems that remain to be solved. For example, the differentiated cells only have a low capability of synthesizing insulin, and furthermore, they show insufficient glucose-responsive insulin (C-peptide) secretion.

Neurogenin 3 (Ngn3) is a known key transcription factor in the development of pancreatic endocrine cells, and the lack of endocrine cells has been confirmed in Ngn3-deficient mice [32]. An experiment using mouse ES cells wherein Ngn3 expression was regulatable showed that the expression of genes associated with pancreatic β -cell development (e.g., NeuroD1 and Nkx2.2) were upregulated upon expression of the Ngn3 gene [33]. This approach induced differentiation, and the resulting insulin-producing cells were C-peptide-positive and glucose responsive. However, the differentiation efficiency was significantly limited with this approach.

The induction of cell differentiation by mimicking the precise molecular course of pancreatic development has been of immense interest in recent years (Fig. 2). First, Yasunaga et al. [34] reported that culturing ES cells in the presence of activin under serum-free conditions produces gooseoid/Sox17/E-cadherin/CXCR4-positive cells, which are definitive and visceral endoderm cells. D'Amour et al. adapted a similar approach and developed a method for generating pancreatic progenitors from human ES cells in 2006 [35]. However, the efficiency of differentiation into insulin-producing cells was approximately 12% with this approach. These cells were not glucose-responsive, although the insulin content was comparative to that in pancreatic β -cells in adults. The expression levels of genes (e.g., Nkx6.1 and Pdx1) essential for the maintenance of β -cell

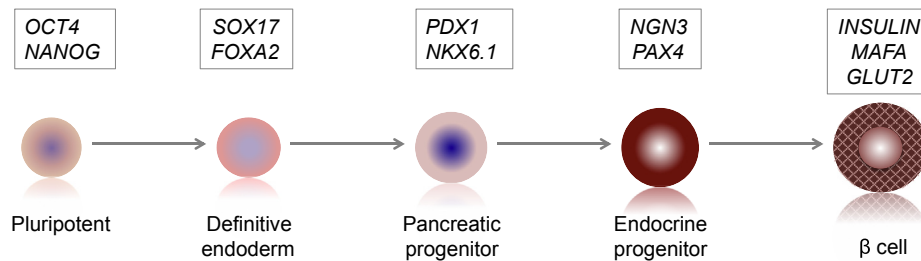


Fig. 2. A schematic representation of the differentiation protocol for insulin secreting β -cells from hES and hiPS cells.

function were extremely low, and this may result in a decreased differentiation efficiency at each step, eventually generating a small number of insulin-producing cells. Furthermore, many cells were not mature, and they produced other hormones. Alternatively, the same group showed that immature progenitor cells were transplanted for the *in vivo* maturation into pancreatic β -cells. This strategy was successful, showing maturation of the pancreatic endoderm derived from human ES cells in mice [36]. Afterwards, culture methods involving step-wise medium replacement have been gradually optimized, and the current major procedure involves a following sequence: induction of embryonic endoderm using activin A plus Wnt3 or PI3K inhibitors in the first step, followed by differentiation into Pdx1-positive pancreatic progenitor cells in the presence of retinoic acid and Noggin. The efficiency of differentiation into Pdx1-positive cells was increased to 80% by this approach. Other agents, such as bone morphogenic protein (BMP) and hedgehog signal inhibitors, have been tested in order to increase the differentiation efficiency into pancreatic progenitor cells. The methods using low molecular compounds discovered by large-scale screening have also been of great interest in recent years. Chen et al. [37] screened 5000 compounds to identify the low molecular compound indolactam V (ILV) as an agent that effectively differentiates hES cells into Pdx-1-positive precursor cells. Furthermore, the same group examined 4000 compounds and identified two compounds that induce the differentiation of human and mouse ES cells into endodermal cells. It was also shown that the addition of ILV after the above differentiation step increases the number of Pdx-1-positive pancreatic precursor cells compared with the method using conventional supplements. Although these progenitor cells can differentiate into insulin-producing cells under the conditions generated using these approaches, the acquisition of the ability to secrete insulin in a glucose-dependent manner remains difficult. Finally, safety (e.g., teratoma formation) and ethical issues need to be addressed before clinical application.

3.2. Induction of pancreatic β -cells from induced pluripotent stem cells

After the discovery of iPS cells, the experimental protocol used to induce the differentiation of mouse [38,39] or human [40–42] iPS cells into insulin-producing cells is almost the same as the one for ES cells [37,43,44], requiring stepwise differentiation into SOX-17-positive, Pdx-1-positive, and then Ngn-3-positive progenitors [43,45–48]. In 2008, for the first time, Tateishi et al. [46] successfully induced pancreatic β -like cells from human iPS (hiPS) cells generated from fibroblasts, demonstrating that in addition to ES cells, iPS cells could be a cellular source for insulin-producing cells (Table 1). However, the differentiation efficiency ranged widely among the iPS cell clones [42,46,48,49]. Later, Hrvatin et al. used 3 different hiPS cell lines, fetal pancreatic cells, and adult insulin positive cells to induce insulin-producing cells. Gene transcription analysis of the cells and adult pancreatic β -cells revealed that the 3 independent hiPS cell lines differentiated into very

similar insulin positive cell populations that are closer to human fetal pancreatic β -cells than to adult pancreatic β -cells [50]. Although capable of producing insulin, human fetal pancreatic β -cells secrete a negligible amount of insulin compared with the adult cells, which has a capacity to respond the blood sugar level. In accordance with these findings, the immature pancreatic β -cells induced from hiPS cells generally lacked glucose responsive insulin secretion and the co-expression of many hormones, including insulin and glucagon [43,49]. In other words, insulin positive cells induced *in vitro* differ from normal adult pancreatic β -cells in that they have not yet differentiated into mature pancreatic β -cells [51]. To realize the clinical application of iPS cells, mature pancreatic β -cells need to be produced stably in large quantities, while maintaining the homogeneity among the differentiated cells. In addition, the induced insulin-producing cells need to survive for a long period of time without forming tumors. After addressing these issues, the superiority of an iPS cell based treatment for diabetes, over the present treatment, should be demonstrated. Furthermore, pancreatic β -cells induced from iPS cells would be useful for not only the establishment of cell differentiation technology but also for the pathological analysis of type 1 and 2 diabetes if the cells are induced from iPS cells established from diabetic patients. In this sense, recent remarkable progress in beta cell maturation strategies is noteworthy, yet to be confirmed their reproducibility [60].

3.3. Direct reprogramming into pancreatic β -cells

As discussed above, the application of stem cells is affected by various factors, such as the efficiency of inducing differentiation into pancreatic β -cells, tumor formation due to contamination of the undifferentiated cells, and the survival of the transplanted cells. Consequently, some studies focus on the production of pancreatic β -cells directly from differentiated cells, by a process called direct reprogramming, bypassing the pluripotent state. In a study using a viral system to express certain genes (such as Ngn3, Pdx1, and MafA), Zhou et al. [52] successfully induced insulin-producing cells from acinar cells in adult mice. The transplantation of these insulin-producing cells led to a reduction in blood glucose levels in diabetic mice, demonstrating the treatment capability of the cells.

Classically, Sosa et al. revealed that the expression of Pax4 led to the successful induction of pancreatic β -cells, and the loss of Pax4 resulted in a decrease in the number of pancreatic β -cells and an increase in the number of α -cells [53]. A study using the pancreatic duct ligation model showed that pancreatic injury resulted in an increased number of pancreatic islet cells because of the trans-differentiation that occurred in the neighboring tissues [54]. In this model, the disappearance of the acinar cells was observed concurrently with an increase in pancreatic ductal cells. According to Xu et al. [55], the ligation of the pancreatic duct induced the reexpression of Ngn3 and the differentiation of the produced endocrine progenitor cells into pancreatic β -cells. However, a recent study showed no change in the number of pancreatic β -cells after

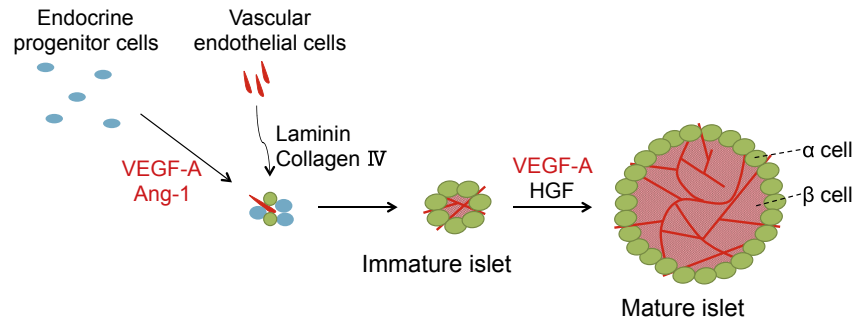


Fig. 3. Interactions between blood vessels and endocrine cells.

pancreatic duct ligation [56,57]. Therefore, further studies are needed to substantially increase the efficiency of *in vivo* induction in order to improve treatment efficacy and to investigate the similarities, such as the functionality, size, morphology, and microstructure, between the induced cells and the mature pancreatic β -cells.

3.4. Generation of pancreatic β -cells using the three-dimensional cell culture method

Various *in vitro* techniques have been used to induce the differentiation of pluripotent stem cells into insulin-producing cells, but none of them have produced cells with functions similar to those of normal pancreatic β -cells. We attribute this to the notion that the induced cells are somehow trapped in an immature stage of differentiation. Therefore, to fully develop into functional pancreatic β -cells, it is essential to establish a differentiation inducing system that accurately reproduces the normal physiological process of differentiation beyond the exposure or transient overexpression of specific proteins.

In recent years, 3D culture techniques have been used to improve the efficiency of stem cell differentiation or to help the cells to attain the last stage of differentiation [58–61]. In fact, the functional capability of the pancreatic islet to secrete insulin declines when the isolated islet is separated into individual cells, suggesting that the cell–cell interactions are essential for the function and maturation of pancreatic β -cells [62]. However, to date, none of the 3D culture methods guide the maturation of β -cells. It is believed that one of the reasons for this is the absence of supporting structures such as blood vessels. The vasculature not just provides nutrients and oxygen to tissues but also contributes to the construction of the 3D tissue architecture with cell polarity and is essential for the differentiation, growth, and maintenance of cells prior to blood perfusion. Engineered pancreatic tissues are, thus, unable to potentiate their functions in the same way as development because their maturation process has been compromised because of the lack of the accompanying vascularization (Fig. 3). Additionally, one might consider the role of other supporting lineages including mesenchyme, immune cells and neuronal cells in proper organogenesis, yet to be characterized.

Therefore, to model the more complex tissue development *in vitro*, there is a need to develop a specific culture by examining communications across different lineages or germ layers. Remarkably, a recently developed culture principle using a “self-condensation” method has enabled the study of aspects of early organogenesis in culture. With this principle, mesenchymal cell-driven condensation on soft substrate generate complex and large (~5 mm in size) tissues comprised of any heterotypic cell populations including stem cell-derived multiple progenitors [63]. The promise of this technology was initially documented by hiPS cell-derived rudimentary liver (liver bud) transplantation grown from

iPS cell derived hepatic endoderm, human endothelial cells and mesenchymal stem cells [63]. By applying this method for enhancing vascularization of the mouse pancreatic β -cells, we successfully generated a pancreatic islet-like tissue with vascular structures and transplanted the tissue into hyperglycemic animals to effectively treat diabetes (Fig. 4) [64]. Unlike the conventional cell differentiation systems, which reproduce the developmental process of the pancreas *in vitro* upon the addition of differentiation inducing factors, the 3D culture system not merely enable a quick tissue vascularization, but activate cell intrinsic developmental program in the presence of optimal supporting cell populations. Based on these findings, we expect that these 3D culture approach will become a basic technique in the establishment of regenerative medicine for pancreatic diseases and for the development of novel treatments for diabetes in the future.

4. Integrating bioengineering technology for clinical transplantation

To establish effective transplantation therapies against diabetes, it is necessary to take full advantage of engineering technologies, especially biomaterials engineering, especially because of the necessity for islet protection from autoimmune destruction. For example, a bioartificial pancreas is made of pancreatic β -cells covered by a semipermeable membrane [65–75] or gel [76–87] to ensure immunoisolation. The immunoisolating semipermeable membrane allows glucose, oxygen, and other low molecular weight nutrients to enter and insulin to diffuse out, while preventing immune cells, high molecular weight antibodies and complement proteins from passing through the membrane. Furthermore, the immunoisolating semipermeable membrane enables the possible use of islet xenografts, which normally provoke an intense immune rejection and often do not survive, thus solving typical transplantation-related challenges, such as immunosuppression and the lack of donor tissues. To date, various bioartificial pancreases have been developed, and they can be largely divided into semipermeable diffusion chamber and encapsulation type pancreatic islets based on the size and method of immunoisolation.

Using diffusion chambers containing pancreatic tissue from newborn rabbits, Gates et al. performed xenograft transplantation and successfully normalized blood glucose levels in diabetic rats [65]. In addition, the pancreatic islet filling a cavity created by immunoisolating semipermeable membranes was transplanted into the peritoneal cavity in a previous study [66–68]. Diffusion chambers are suited for the development and optimization of semipermeable membranes but necessitate some planning and ingenuity for encapsulation and also require measures to prevent aggregation and necrosis of the islet floating inside the chamber. As a modified type of this approach, blood is perfused through an arteriovenous shunt made of semipermeable membranes, and the

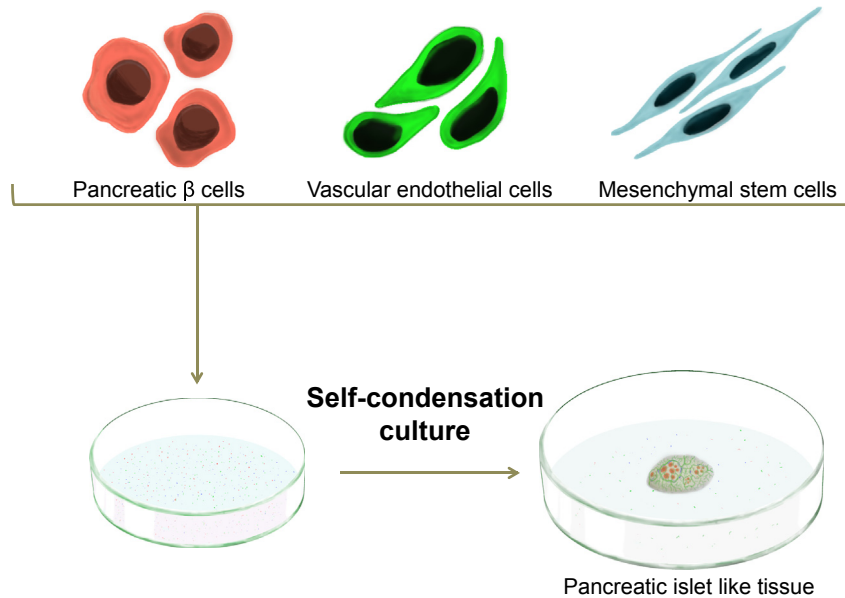


Fig. 4. Generation of islet-like structure with human vascular networks.

islets are placed into the surrounding tissue [69,70]. Although the transplantation is relatively easy, the method requires anticoagulation therapy because of a high risk of thrombus formation and is not suited for long-term use.

Encapsulated islets are currently the most anticipated method of creating an artificial pancreatic islet [71,76]. In this method, islets are enclosed by membranes and gels, such as agarose [77,78], and contain alginate [72] as the primary component. Depending on the size, the encapsulated islets are classified as microencapsulated or macroencapsulated cells. For microencapsulation, the islets are enclosed in gel beads measuring several hundred microns in diameter [79], offering a large surface area and favorable permeation compared with the macroencapsulated cells. However, beads that do not contain islets, which are produced during the production process, should be removed. In addition, when adverse events occur, the safety of the system is unfavorable because it is difficult to recover the beads from the abdominal cavity or another body cavity. Therefore, research is currently underway to develop much smaller capsules for transplantation into subcutaneous or muscle tissue, which involves safe and minimal invasive procedures or injection into the portal vein, which is currently the target site of islet transplantation. Another advantage of using smaller capsules is the shorter distance between the islet and the outside environment, thereby enhancing membrane permeability and the maintenance of cellular functions. For example, Teramura et al. successfully wrapped pancreatic islets using extremely thin membranes that they produced by binding polyvinyl alcohol (PVA) to polyethylene glycol (PEG)-lipid conjugates that are immobilized on the surface of islets through the interaction with the lipid layer of the cell membrane [73,74]. By taking advantage of the self-assembling property of streptavidin and biotin conjugated to poly(L-lysine)-PEG, Wilson et al. developed a novel technique to wrap pancreatic islets in thin membranes and showed that the survival rate and amount of insulin secretion of these cells were similar to those of normal pancreatic islets [75], suggesting that the cells are suitable for injection into the portal vein in mice. However, because the physicochemical and biological durability and the immunoisolation properties of the thin membrane remain largely unknown, further studies are necessary to realize its clinical application. To overcome the drawback of the microencapsulated

cells, that is, their inability to be recovered, research has focused on macroencapsulation, in which islets are wrapped in a gel large enough to handle macroscopically. Compared with microencapsules, the relative surface area is small, and the permeability is somehow unfavorable. However, with ingenious alterations in the graft sites, macrocapsules may be suitable for clinical application because they can be retrieved if adverse events occur or if their efficacy disappears, thus ensuring safety. In a previous study using mice and rats [80–83], pancreatic islets placed in a tube made of a PVA gel reinforced by a mesh was transplanted to treat diabetes. In another study, by taking advantage of the physicochemical properties of PVA dissolved in water, that is, the formation of fine crystalline structures and the polymerization at low temperatures, and by integrating these properties into the frozen storage of pancreatic islets, the authors formed a sheet of an aqueous PVA solution mixed with cells. Then, by freezing and thawing the sheet, the authors successfully microencapsulated the pancreatic islet cells using the PVA gel, producing effective islet grafts [84–87]. These encapsulation techniques will be beneficial in terms of measures to reduce health risks, such as tumor formation, when it is time to transplant pancreatic islet-like tissues differentiated from the stem cells, such as ES or iPS cells, in the future.

Recent advances in nanotechnology have enabled the regulation of cells through the scaffolds made of biodegradable polymers, and research to create homogeneous and dense cell cultures using scaffold materials has intensified. Okano et al. proposed the production of a sheet of tissue made of a single or multiple layers of cells [88]. To achieve this, a temperature-responsive polymer called poly-N-isopropylacrylamide (PIPAAm), which changes its hydrophilicity with temperature, was immobilized evenly on the inside surface of a culture dish at the nanoscale level. The temperature is then changed to modify the hydrophilicity of PIPAAm and the adhesion of the cells growing on this special dish. Cells grow to confluence on the base of this temperature-responsive polymer at 37 °C, but a sheet of cells comes off the base material when the temperature is dropped to ≤ 32 °C. These sheets are void of chemical or physical damage and maintain extracellular matrix and adhesion molecules, enhancing their survival in tissues. Sheets of pancreatic islets can be transplanted to humans to perform the function of the pancreatic islet for a long time and would eventually turn into an artificial organ. By

reforming the pancreatic islet and producing sheets of islet cells, Shimizu et al. successfully transplanted the islet cells even in a space with a poor blood supply, such as subcutaneous tissue [89,90]. Therefore, they addressed the problems associated with pancreatic islet transplantation, namely, instant blood-mediated reaction (IBMIR) and graft failure because of inflammation in the area supplied by the portal vein due to ischemia induced by the islet. The clinical application of the pancreatic islet sheets separated from the culture dish is expected to be highly effective because these sheets can be transplanted into various tissues and organs after individualizing the size and shape of the sheets to meet the physiological functions of each patient [91–95].

In the field of biomaterials, research and development has conventionally been performed to create materials that are biocompatible and quickly adapt to the human body, without stimulating the innate inflammatory and immune systems, and thus, avoiding recognition or rejection by the body. However, in recent years, research has focused on developing biomaterials that proactively reach out to the body. In mice, Shapiro et al. first created a subcutaneous site densely packed with blood vessels by subcutaneously imbedding a device containing pro-angiogenic factors, and then, by removing the device, they were able to transform the site into a capillary bed with a space for transplantation [96]. These beds enable the tissues to promote long-term survival and functional maintenance of cells and tissues transplanted in the space. Other recent advance is to freely fabricate the shape and size of tissues by employing our cell-laden fibres technology [97]. Onoe et al. showed the promise of highly handleable characteristic of their fibres for the encapsulation of the murine islets. Thus, combination of the above described transplantation approaches, which is minimally invasive and offers removable grafts, would improve the applicability to severely diabetic patients who cannot be otherwise treated.

5. Conclusion

We have summarized the state-of-art of β -cell induction approaches. However, it needs to be emphasized that previous studies have not established a fully reproducible technique that can faithfully cure diabetes through the generation of mature pancreatic β -cells. Alternatively, pancreatic progenitor transplantation, i.e., *in vivo* maturation approaches, hold promise by making a retrievable graft, as ViaCyte just started the clinical trial recently. Other progress on-going would be a 3-D culture based approaches to overcome the previous limitations of flat plate culture based approaches with the use of self-organizing organoid or organ bud. Together, these efforts one day offer hope for the complete cure for severe diabetes and will yield novel regenerative approaches for the treatment of end-stage organ failure.

Conflict of interest

Drs. Takebe and Taniguchi have served on a scientific advisory boards for Healios Inc.

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References

- Oliver-Krasinski JM, Stoffers DA. On the origin of the beta cell. *Genes Dev* 2008;22(15):1998–2021.
- Slack J. Origin of stem cells in organogenesis. *Science* 2008;322(5907):1498–501.
- Zaret KS, Grompe M. Generation and regeneration of cells of the liver and pancreas. *Science* 2008;322(5907):1490–4.
- Kim SK, Hebrok M. Interacellular signals regulating pancreas development and function. *Genes Dev* 2001;15(2):111–27.
- Jørgensen MC, Ahnfelt-Rønne J, Hald J, Madsen OD, Serup P, Hecksher-Sørensen J. An illustrated review of early pancreas development in the mouse. *Endocr Rev* 2007;28(6):685–705.
- Pan FC, Wright C. Pancreas organogenesis: from bud to plexus to gland. *Dev Dyn* 2011;240(3):530–65.
- Katsumoto K, Kume S. Endoderm and mesoderm reciprocal signaling mediated by CXCL12 and CXCR4 regulates the migration of angioblasts and establishes the pancreatic fate. *Development* 2011;138(10):1947–55.
- Yoshida T, Murata K, Shiraki N, Kume K, Kume S. Analysis of gene expressions of embryonic stem-derived Pdx1-expressing cells: Implications of genes involved in pancreas differentiation. *Dev Growth Differ* 2009;51(4):463–72.
- Jonsson J, Carlsson L, Edlund T, Edlund H. Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* 1994;371(6498):606–9.
- Offield MF, Jetton TL, Labosky PA, Ray M, Stein RW, Magnuson MA, et al. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* 1996;122(3):983–95.
- Apelqvist Å, Ahlgren U, Edlund H. Sonic hedgehog directs specialised mesoderm differentiation in the intestine and pancreas. *Curr Biol* 1997;7(10):801–4.
- Hebrok M, Kim SK, Melton DA. Notochord repression of endodermal Sonic hedgehog permits pancreas development. *Genes Dev* 1998;12(11):1705–13.
- Hebrok M, Kim SK, St Jacques B, McMahon AP, Melton DA. Regulation of pancreas development by hedgehog signaling. *Development* 2000;127(22):4905–13.
- Kim SK, Hebrok M, Melton DA. Notochord to endoderm signaling is required for pancreas development. *Development* 1997;124(21):4243–52.
- Kim SK, Melton DA. Pancreas development is promoted by cyclopamine, a hedgehog signaling inhibitor. *Proc Natl Acad Sci U. S. A* 1998;95(22):13036–41.
- Bayha E, Jørgensen MC, Serup P, Grapin-Botton A. Retinoic acid signaling organizes endodermal organ specification along the entire antero-posterior axis. *PLoS One* 2009;4(6):e5845.
- Stafford D, White RJ, Kinkel MD, Linville A, Schilling TF, Prince VE. Retinoids signal directly to zebrafish endoderm to specify insulin-expressing β -cells. *Development* 2006;133(5):949–56.
- Kim SK, Hebrok M, Li E, Oh SP, Schrewe H, Harmon EB, et al. Activin receptor patterning of foregut organogenesis. *Genes Dev* 2000;14(15):1866–71.
- Hebrok M. Hedgehog signaling in pancreas development. *Mech Dev* 2003;120(1):45–57.
- Lammert E, Cleaver O, Melton D. Induction of pancreatic differentiation by signals from blood vessels. *Science* 2001;294(5542):564–7.
- Lammert E, Cleaver O, Melton D. Role of endothelial cells in early pancreas and liver development. *Mech Dev* 2003;120(1):59–64.
- Konstantinova I, Lammert E. Microvascular development: learning from pancreatic islets. *Bioessays* 2004;26(10):1069–75.
- Yoshitomi H, Zaret KS. Endothelial cell interactions initiate dorsal pancreas development by selectively inducing the transcription factor Ptf1a. *Development* 2004;131(4):807–17.
- Landsman L, Nijagal A, Whitchurch TJ, VanderLaan RL, Zimmer WE, MacKenzie TC, et al. Pancreatic mesenchyme regulates epithelial organogenesis throughout development. *PLoS Biol* 2011;9(9):1904.
- Matsunari H, Nagashima H, Watanabe M, Umeyama K, Nakano K, Nagaya M, et al. Blastocyst complementation generates exogenous pancreas *in vivo* in apancreatic cloned pigs. *Proc Natl Acad Sci U S A* 2013;110(12):4557–62.
- Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* 2001;292(5520):1389–94.
- Afrikanova I, Yebra M, Simpkinson M, Xu Y, Hayek A, Montgomery A. Inhibitors of Src and focal adhesion kinase promote endocrine Specification: Impact on the derivation of β -cells from human pluripotent stem cells. *J Biol Chem* 2011;286(41):36042–52.
- Moritoh Y, Yamato E, Yasui Y, Miyazaki S, Miyazaki J-i. Analysis of insulin-producing cells during *in vitro* differentiation from feeder-free embryonic stem cells. *Diabetes* 2003;52(5):1163–8.
- Miyazaki S, Yamato E, Miyazaki J-i. Regulated expression of pdx-1 promotes *in vitro* differentiation of insulin-producing cells from embryonic stem cells. *Diabetes* 2004;53(4):1030–7.
- Hori Y, Rulifson IC, Tsai BC, Heit JJ, Cahoy JD, Kim SK. Growth inhibitors promote differentiation of insulin-producing tissue from embryonic stem cells. *Proc Natl Acad Sci U S A* 2002;99(25):16105–10.
- Bernardo AS, Cho CHH, Mason S, Docherty HM, Pedersen RA, Vallier L, et al. Biphasic induction of Pdx1 in mouse and human embryonic stem cells can mimic development of pancreatic β -cells. *Stem Cells* 2009;27(2):341–51.

- [32] Gradwohl G, Dierich A, LeMeur M, Guillemot F. neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proc Natl Acad Sci U S A* 2000;97(4):1607–11.
- [33] Serafimidis I, Rakatzi I, Episkopou V, Gouti M, Gavalas A. Novel effectors of directed and Ngn3-mediated differentiation of mouse embryonic stem cells into endocrine pancreas progenitors. *Stem Cells* 2008;26(1):3–16.
- [34] Yasunaga M, Tada S, Torikai-Nishikawa S, Nakano Y, Okada M, Jakt LM, et al. Induction and monitoring of definitive and visceral endoderm differentiation of mouse ES cells. *Nat Biotechnol* 2005;23(12):1542–50.
- [35] D'Amour KA, Bang AG, Eliazer S, Kelly OG, Agulnick AD, Smart NG, et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol* 2006;24(11):1392–401.
- [36] Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells *in vivo*. *Nat Biotechnol* 2008;26(4):443–52.
- [37] Chen S, Borowiak M, Fox JL, Maehr R, Osafune K, Davidow L, et al. A small molecule that directs differentiation of human ESCs into the pancreatic lineage. *Nat Chem Biol* 2009;5(4):258–65.
- [38] Alipio Z, Liao W, Roemer EJ, Waner M, Fink LM, Ward DC, et al. Reversal of hyperglycemia in diabetic mouse models using induced-pluripotent stem (iPS)-derived pancreatic β -like cells. *Proc Natl Acad Sci U S A* 2010;107(30):13426–31.
- [39] Jeon K, Lim H, Kim J-H, Thuan NV, Park SH, Lim Y-M, et al. Differentiation and transplantation of functional pancreatic beta cells generated from induced pluripotent stem cells derived from a type 1 diabetes mouse model. *Stem Cells Dev* 2012;21(14):2642–55.
- [40] Kunisada Y, Tsubooka-Yamazoe N, Shoji M, Hosoya M. Small molecules induce efficient differentiation into insulin-producing cells from human induced pluripotent stem cells. *Stem Cell Res* 2012;8(2):274–84.
- [41] Ohmine S, Squillace KA, Hartjes KA, Deeds MC, Armstrong AS, Thatava T, et al. Reprogrammed keratinocytes from elderly type 2 diabetes patients suppress senescence genes to acquire induced pluripotency. *Aging (Albany NY)* 2012;4(1):60.
- [42] Thatava T, Nelson TJ, Edukulla R, Sakuma T, Ohmine S, Tonne JM, et al. Indolactam V/GLP-1-mediated differentiation of human iPS cells into glucose-responsive insulin-secreting progeny. *Gene Ther* 2011;18(3):283–93.
- [43] Zhang D, Jiang W, Liu M, Sui X, Yin X, Chen S, et al. Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. *Cell Res* 2009;19(4):429–38.
- [44] Jiang J, Au M, Lu K, Eshpeter A, Korbitt G, Fisk G, et al. Generation of insulin-producing islet-like clusters from human embryonic stem cells. *Stem Cells* 2007;25(8):1940–53.
- [45] Maehr R, Chen S, Snitow M, Ludwig T, Yagasaki L, Goland R, et al. Generation of pluripotent stem cells from patients with type 1 diabetes. *Proc Natl Acad Sci U S A* 2009;106(37):15768–73.
- [46] Tateishi K, He J, Taranova O, Liang G, D'Alessio AC, Zhang Y. Generation of insulin-secreting islet-like clusters from human skin fibroblasts. *J Biol Chem* 2008;283(46):31601–7.
- [47] Hua H, Shang L, Martinez H, Freeby M, Gallagher MP, Ludwig T, et al. iPSC-derived β cells model diabetes due to glucokinase deficiency. *J Clin Invest* 2013;123(7):3146.
- [48] Thatava T, Kudva YC, Edukulla R, Squillace K, De Lamo JG, Khan YK, et al. Intrapatient variations in type 1 diabetes-specific iPS cell differentiation into insulin-producing cells. *Mol Ther* 2013;21(1):228–39.
- [49] Nostro MC, Sarangi F, Ogawa S, Holtzinger A, Corneo B, Li X, et al. Stage-specific signaling through TGF β family members and WNT regulates patterning and pancreatic specification of human pluripotent stem cells. *Development* 2011;138(5):861–71.
- [50] Hrvatin S, O'Donnell CW, Deng F, Millman JR, Pagliuca FW, Dilorio P, et al. Differentiated human stem cells resemble fetal, not adult, β cells. *Proc Natl Acad Sci U S A* 2014;111(8):3038–43.
- [51] Teo AKK, Wagers AJ, Kulkarni RN. New opportunities: harnessing induced pluripotency for discovery in diabetes and metabolism. *Cell Metab* 2013;18(6):775–91.
- [52] Zhou Q, Melton DA. Extreme makeover: converting one cell into another. *Cell Stem Cell* 2008;3(4):382–8.
- [53] Sosa-Pineda B, Chowdhury K, Torres M, Oliver G, Gruss P. The Pax4 gene is essential for differentiation of insulin-producing β cells in the mammalian pancreas. *Nature* 1997;386(6623):399–402.
- [54] Wang R, Klöppel G, Bouwens L. Duct-to islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats. *Diabetologia* 1995;38(12):1405–11.
- [55] Xu X, D'Hoker J, Stange G, Bonne S, De Leu N, Xiao X, et al. β cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell* 2008;132(2):197–207.
- [56] Chintinne M, Stangé G, Denys B, Ling Z, Pipeleers D. Beta cell count instead of beta cell mass to assess and localize growth in beta cell population following pancreatic duct ligation in mice. *PLoS One* 2012;7(8): e43959-e.
- [57] Rankin MM, Wilbur CJ, Rak K, Shields EJ, Granger A, Kushner JA. β -Cells are not generated in pancreatic duct Ligation-Induced injury in adult mice. *Diabetes* 2013;62(5):1634–45.
- [58] Wang X, Ye K. Three-dimensional differentiation of embryonic stem cells into islet-like insulin-producing clusters. *Tissue Eng Part A* 2009;15(8):1941–52.
- [59] Saito H, Takeuchi M, Chida K, Miyajima A. Generation of glucose-responsive functional islets with a three-dimensional structure from mouse fetal pancreatic cells and iPS cells *in vitro*. *PLoS one* 2011;6(12):e28209.
- [60] Pagliuca FW, Millman JR, Gürtler M, Segel M, Van Dervort A, Ryu JH, et al. Generation of functional human pancreatic β cells *in vitro*. *Cell* 2014;159(2):428–39.
- [61] Toyoda T, Mae S-I, Tanaka H, Kondo Y, Funato M, Hosokawa Y, et al. Cell aggregation optimizes the differentiation of human ESCs and iPSCs into pancreatic bud-like progenitor cells. *Stem Cell Res* 2015;14(2):185–97.
- [62] Kojima N, Takeuchi S, Sakai Y, editors. Engineering of pseudoislets: effect on insulin secretion activity by cell number, cell population, and microchannel networks. *Transplant proc*, vol. 46; 2014. p. 1161–5. 4.
- [63] Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 2013;499(7459):481–4.
- [64] Takebe T, Enomura M, Yoshizawa E, Kimura M, Koike H, Ueno Y, et al. Vascularized and complex organ buds from diverse tissues via mesenchymal cell-driven condensation. *Cell Stem Cell* 2015;16(5):556–65.
- [65] Gates R, Lazarus N. Reversal of streptozotocin-induced diabetes in rats by intraperitoneal implantation of encapsulated neonatal rabbit pancreatic tissue. *Lancet* 1977;310(8051):1257–9.
- [66] Lanza RP, Borland KM, Lodge P, Carretta M, Sullivan SJ, Muller TE, et al. Treatment of severely diabetic pancreatctomized dogs using a diffusion-based hybrid pancreas. *Diabetes* 1992;41(7):886–9.
- [67] Edamura K, Itakura S, Nasu K, Iwami Y, Ogawa H, Sakai N, et al. Xenotransplantation of porcine pancreatic endocrine cells to total pancreatectomized dogs. *J Vet Med Sci* 2003;65(5):549–56.
- [68] Maratos E, Taub R, Bramis J. Amelioration of streptozotocin-induced diabetes in mice by the implantation of pancreatic islets in diffusion chambers. *Mt Sinai J Med N Y* 1976;43(4):415.
- [69] Reach G, Jaffrin MY, Desjeux J-F. A u-shaped bioartificial pancreas with rapid glucose-insulin kinetics: In vitro evaluation and kinetic modelling. *Diabetes* 1984;33(8):752–61.
- [70] Maki T, Cs Ubhi, Sanchez-Farpon H, Sj Sullivan, Borland K, Te Muller, et al. Successful treatment of diabetes with the biohybrid artificial pancreas in dogs. *Transplantation* 1991;51(1):43–50.
- [71] Calafiore R, Basta G, Luca G, Lemmi A, Montanucci MP, Calabrese G, et al. Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes. *Diabetes Care* 2006;29(1):137–8.
- [72] Sun AM, O'shea GM, Goosen MF. Injectable microencapsulated islet cells as a bioartificial pancreas. *Appl Biochem Biotechnol* 1984;10:87–99.
- [73] Hill RS, Cruise GM, Hager SR, Lamberti FV, Yu X, Garufis CL, et al. Immunolocalization of adult porcine islets for the treatment of diabetes mellitus. *Ann N Y Acad Sci* 1997;831(1):332–43.
- [74] Teramura Y, Kaneda Y, Iwata H. Islet-encapsulation in ultra-thin layer-by-layer membranes of poly (vinyl alcohol) anchored to poly (ethylene glycol)-lipids in the cell membrane. *Biomaterials* 2007;28(32):4818–25.
- [75] Wilson JT, Cui W, Chaikof EL. Layer-by-layer assembly of a conformal nanothin PEG coating for intraportal islet transplantation. *Nano Lett* 2008;8(7):1940–8.
- [76] Tuch BE, Keogh GW, Williams LJ, Wu W, Foster JL, Vaithilingam V, et al. Safety and viability of microencapsulated human islets transplanted into diabetic humans. *Diabetes Care* 2009;32(10):1887–9.
- [77] Iwata H, Takagi T, Amemiya H, editors. Agarose microcapsule applied in islet xenografts (hamster to mouse). *Transplant proc*, vol. 24; 1992. p. 952. 3.
- [78] Iwata H, Amemiya H, Matsuda T, Takano H, Hayashi R, Akutsu T. Evaluation of microencapsulated islets in agarose gel as bioartificial pancreas by studies of hormone secretion in culture and by xenotransplantation. *Diabetes* 1989;38:224–5.
- [79] Sun AM. Microencapsulation of pancreatic islet cells: a bioartificial endocrine pancreas. *Methods Enzymol* 1988;137:575–80.
- [80] Inoue K, Fujisato T, Gu Y, Burczak K, Sumi S, Kogire M, et al. Experimental hybrid islet transplantation: application of polyvinyl alcohol membrane for entrapment of islets. *Pancreas* 1992;7(5):562–8.
- [81] Inoue K, Gu Y, Hayashi H, Shinohara S, Aung T, Tun T, et al., editors. Pig-to-rat xenotransplantation with mesh-reinforced polyvinyl alcohol bag: efficacy of agarose gel. *Transplant proc*, 28; 1996. p. 1422–3. 3.
- [82] Wang W, Gu Y, Tabata Y, Miyamoto M, Hori H, Nagata N, et al. Reversal of diabetes in mice by xenotransplantation of a bioartificial pancreas in a prevascularized subcutaneous site. *Transplantation* 2002;73(1):122–9.
- [83] Wang W, Gu Y, Hori H, Sakurai T, Hiura A, Sumi S, et al. Subcutaneous transplantation of macroencapsulated porcine pancreatic endocrine cells normalizes hyperglycemia in diabetic mice. *Transplantation* 2003;76(2):290–6.
- [84] Qi M, Gu Y, Sakata N, Kim D, Shirouzu Y, Yamamoto C, et al. PVA hydrogel sheet macroencapsulation for the bioartificial pancreas. *Biomaterials* 2004;25(27):5885–92.
- [85] Sakata N, Gu Y, Qi M, Yamamoto C, Hiura A, Sumi S, et al. Effect of rat-to-mouse bioartificial pancreas xenotransplantation on diabetic renal damage and survival. *Pancreas* 2006;32(3):249–57.
- [86] Qi Z, Shen Y, Yanai G, Yang K, Shirouzu Y, Hiura A, et al. The *in vivo* performance of polyvinyl alcohol macro-encapsulated islets. *Biomaterials* 2010;31(14):4026–31.
- [87] Qi Z, Yamamoto C, Imori N, Kinukawa A, Yang K-C, Yanai G, et al. Immunolocalization effect of polyvinyl alcohol (PVA) macroencapsulated islets in type 1 diabetes therapy. *Cell Transpl* 2012;21(2–3):525–34.

- [88] Yamada N, Okano T, Sakai H, Karikusa F, Sawasaki Y, Sakurai Y. Thermo-responsive polymeric surfaces; control of attachment and detachment of cultured cells. *Die Makromol Chem Rapid Commun* 1990;11(11):571–6.
- [89] Shimizu H, Ohashi K, Utoh R, Ise K, Gotoh M, Yamato M, et al. Bioengineering of a functional sheet of islet cells for the treatment of diabetes mellitus. *Biomaterials* 2009;30(30):5943–9.
- [90] Saito T, Ohashi K, Utoh R, Shimizu H, Ise K, Suzuki H, et al. Reversal of diabetes by the creation of neo-islet tissues into a subcutaneous site using islet cell sheets. *Transplantation* 2011;92(11):1231–6.
- [91] Yamato M, Utsumi M, Kushida A, Konno C, Kikuchi A, Okano T. Thermo-responsive culture dishes allow the intact harvest of multilayered keratinocyte sheets without dispase by reducing temperature. *Tissue Eng* 2001;7(4):473–80.
- [92] Kushida A, Yamato M, Konno C, Kikuchi A, Sakurai Y, Okano T. Temperature-responsive culture dishes allow nonenzymatic harvest of differentiated Madin-Darby Canine Kidney (MDCK) cell sheets. *J Biomed Mater Res* 2000;51(2):216–23.
- [93] Harimoto M, Yamato M, Hirose M, Takahashi C, Isoi Y, Kikuchi A, et al. Novel approach for achieving double-layered cell sheets co-culture: overlaying endothelial cell sheets onto monolayer hepatocytes utilizing temperature-responsive culture dishes. *J Biomed Mater Res* 2002;62(3):464–70.
- [94] Sakaguchi K, Shimizu T, Horaguchi S, Sekine H, Yamato M, Umezumi M, et al. *In vitro* engineering of vascularized tissue surrogates. *Sci Rep* 2013;3.
- [95] Sekine H, Shimizu T, Sakaguchi K, Dobashi I, Wada M, Yamato M, et al. In vitro fabrication of functional three-dimensional tissues with perfusable blood vessels. *Nat Commun* 2013;4:1399.
- [96] Pepper AR, Gala-Lopez B, Pawlick R, Merani S, Kin T, Shapiro AJ. A prevascularized subcutaneous device-less site for islet and cellular transplantation. *Nat Biotechnol* 2015;33(5):518–23.
- [97] Onoe H, Okitsu T, Itou A, Kato-Negishi M, Gojo R, Kiriya D, et al. Metre-long cell-laden microfibrils exhibit tissue morphologies and functions. *Nat Mater* 2013;12:584–90.